Comparing Haemophilus influenzae type b conjugate vaccine schedules: a systematic review and meta-analysis of vaccine trials

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Abstract: BACKGROUND The optimal schedule and the need for a booster dose are unclear for Haemophilus influenzae type b (Hib) conjugate vaccines. We systematically reviewed relative effects of Hib vaccine schedules. METHODS We searched 21 databases to May 2010 or June 2012 and selected randomized controlled trials or quasi-randomized controlled trials that compared different Hib schedules (3 primary doses with no booster dose [3p+0], 3p+1 and 2p+1) or different intervals in primary schedules and between primary and booster schedules. Outcomes were clinical efficacy, nasopharyngeal carriage and immunological response. Results were combined in random-effects meta-analysis. RESULTS Twenty trials from 15 countries were included; 16 used vaccines conjugated to tetanus toxoid (polyribosylribitol phosphate conjugated to tetanus toxoid). No trials assessed clinical or carriage outcomes. Twenty trials examined immunological outcomes and found few relevant differences. Comparing polyribosylribitol phosphate conjugated to tetanus toxoid 3p+0 with 2p+0, there was no difference in seropositivity at the 1.0 g/mL threshold by 6 months after the last primary dose (combined risk difference -0.02; 95% confidence interval: -0.10, 0.06). Only small differences were seen between schedules starting at different ages, with different intervals between primary doses, or with different intervals between primary and booster doses. Individuals receiving a booster were more likely to be seropositive than those at the same age who did not. CONCLUSIONS There is no clear evidence from trials that any 2p+1, 3p+0 or 3p+1 schedule of Hib conjugate vaccine is likely to provide better protection against Hib disease than other schedules. Until more data become available, scheduling is likely to be determined by epidemiological and programmatic considerations in individual settings.

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Comparing *Haemophilus influenzae* Type b Conjugate Vaccine Schedules

A Systematic Review and Meta-analysis of Vaccine Trials

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Background: The optimal schedule and the need for a booster dose are unclear for *Haemophilus influenzae* type b (Hib) conjugate vaccines. We systematically reviewed relative effects of Hib vaccine schedules.

Methods: We searched 21 databases to May 2010 or June 2012 and selected randomized controlled trials or quasi-randomized controlled trials that compared different Hib schedules (3 primary doses with no booster dose [3p+0], 3p+1 and 2p+1) or different intervals in primary schedules and between primary and booster schedules. Outcomes were clinical efficacy, nasopharyngeal carriage and immunological response. Results were combined in random-effects meta-analysis.

Results: Twenty trials from 15 countries were included; 16 used vaccines conjugated to tetanus toxoid (polyribosylribitol phosphate conjugated to tetanus toxoid). No trials assessed clinical or carriage outcomes. Twenty trials examined immunological outcomes and found few relevant differences. Comparing polyribosylribitol phosphate conjugated to tetanus toxoid 3p+0 with 2p+0, there was no difference in seropositivity at the 1.0 μg/mL threshold by 6 months after the last primary dose (combined risk difference −0.02; 95% confidence interval: −0.10, 0.06). Only small differences were seen between schedules starting at different ages, with different intervals between primary doses, or with different intervals between primary and booster doses. Individuals receiving a booster were more likely to be seropositive than those at the same age who did not.

Conclusions: There is no clear evidence from trials that any 2p+1, 3p+0 or 3p+1 schedule of Hib conjugate vaccine is likely to provide better protection against Hib disease than other schedules. Until more data become available, scheduling is likely to be determined by epidemiological and programmatic considerations in individual settings.

Key Words: *Haemophilus influenzae* type b conjugate vaccine, vaccine schedules, systematic review, meta-analysis

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Haemophilus influenzae type b (Hib) conjugate vaccines have led to large reductions in the incidence of invasive Hib disease, including meningitis and pneumonia, in countries that include them in their routine immunization schedule.[1] Nevertheless, there are still more than 8 million cases of severe Hib disease worldwide annually in children under 5 years.[2] Conjugate vaccines in use in 2012 contained Hib capsular polysaccharide (polyribosylribitol phosphate [PRP]) conjugated to the nontoxic CRM197 variant of diphtheria toxin (PRP-HbOC), meningococcal outer membrane protein (PRP-OMP), or most commonly tetanus toxoid (PRP-T).[1]

Countries are faced with decisions about optimal schedules for vaccines recommended for infants. The 2006 World Health Organization position paper on Hib conjugate vaccines states that a 3-dose schedule can be used with 1 to 2 months between doses, starting as young as 6 weeks.[3] The position paper does not explicitly recommend a booster dose but states that if given it should be at 12–18 months of age. In 2012, most countries using Hib vaccine used a 3-dose primary schedule with no booster dose (3p+0 schedule). Some countries, mainly in Europe and the Americas, added a booster dose to the 3-dose primary schedule (3p+1 schedule) whereas other countries, mainly in Europe, used schedules with 2 primary doses and a booster (2p+1 schedule).[4] Variation in Hib vaccination schedules reflects not only differences in the historical scheduling of childhood vaccines, setting-specific epidemiology, existing health service infrastructure and coadministered vaccines but also uncertainties about the optimal number of primary doses, the interval between doses in the primary schedule and the need for a booster dose.[5] Whilst the clinical efficacy of Hib conjugate vaccines has been summarized,[6–9] there have been no systematic reviews summarizing immunological, carriage and clinical outcomes from trials making head-to-head comparisons of different Hib vaccine schedules.

Here we systematically review the evidence from randomized controlled trials (RCTs) or quasi-randomized trials about the relative effects of 2p+0, 3p+0, 2p+1 and 3p+1 schedules and the effects of different timing of Hib conjugate vaccine doses.

METHODS

The review process followed a protocol, which was completed before starting the review (Supplemental Digital Content 1, http://links.lww.com/INF/B612). Minor amendments were made after the review started, and these are recorded in the protocol document. We report here results for the head-to-head comparisons of Hib conjugate vaccine schedules described in the protocol. Comparisons of Hib schedules to no Hib vaccination will be reported elsewhere.

Study Identification

The literature search covered 21 electronic databases from the earliest citation until May 2010. There were 5 databases of published articles (AIM, Cochrane Library, LILACs, IndMED, Medline), 3 trial registries, 11 vaccine manufacturer

databases and 2 regulatory authority websites. Search strategies included terms for "Hib" and "conjugate vaccine" adapted for each search engine (Supplemental Digital Content 2, http://links.lww.com/INF/B613). In June 2012, the Medline search was updated, using a filter to identify RCTs (2012 search only), and the AIM, CENTRAL, LILACs and IndMED searches were updated using the 2010 search strategy. Eligible trial registrations found in the 2010 search were also checked for new publications in June 2012.

Study Selection

Studies were considered eligible if they were randomized or quasi-randomized (eg, allocated according to date of birth) and examined children vaccinated with PRP-T, PRP-OMP or PRP-HbOC at less than 6 years of age. Trials were eligible if they assigned participants to the following comparisons: 3p+0 vs. 2p+0; 3p+0 vs. 2p+1; 3p+1 vs. 2p+1; 3p+1 vs. 3p+0. We also included studies that compared different intervals between doses and different ages at the start of the primary schedule. We excluded studies where both the schedule and the PRP-conjugated molecule differed between available comparison groups so that no comparisons within the trial assessed the effect of schedule differences alone.

Outcomes included invasive Hib disease as a combined outcome or separate diagnoses of Hib meningitis, pneumonia due to any cause, Hib pneumonia, epiglottitis, nasopharyngeal carriage of Hib, seropositivity after vaccination or geometric mean concentration (GMC) of PRP antibody. Seropositivity was defined by IgG antibody levels measured by enzyme-linked immunoassay or Farr-type radio-assay at threshold values of 0.15 and 1.0 μg/mL.[10] Only systematically collected clinical outcomes were considered eligible.

Each title and abstract was screened for eligibility by 2 independent reviewers. The full texts of abstracts assessed by 1 or both reviewers to be potentially eligible were then screened for eligibility by 2 reviewers. Data were extracted on to a structured piloted form (available on request). Data were extracted by 2 independent reviewers, and differences were resolved by consensus. Items extracted included trial characteristics, outcomes, potential sources of heterogeneity and the risk of bias in individual trials.[11] The risk of bias was assessed by examining trial features including the adequacy of random sequence generation, adequacy of allocation concealment, the use of outcome assessor blinding and the type of analysis.[12,13] Analysis types included modified intention-to-treat (mITT) and per-protocol (PP). Modified intention-to-treat is used to describe analyses that included all randomized (or assigned) participants who had outcome data available with the possible exclusion of those who received no doses of vaccine, and PP is used to describe those that additionally excluded individuals with other protocol violations. We did not contact authors to obtain additional information.

Analysis

We combined data statistically, where appropriate, using DerSimonian and Laird random-effects meta-analysis[14] in STATA version 12 (StataCorp LP, College Station, TX). Between-trial heterogeneity was described using the I^2 statistic, where values below 25% represent low heterogeneity, up to 50% moderate heterogeneity, up to 75% severe heterogeneity and more than 75% very severe heterogeneity.[15] Where multiple intervention groups (or "trial arms") were available within a trial to make a comparison of 2 schedules, we compared the groups that were most similar except for the difference in schedule. The decision about intervention groups to compare was made by 2 senior reviewers (N.L. and P.S.) without reference to trial results. For immunological outcomes, and for both the 1.0 and 0.15 ug/mL thresholds, we calculated the difference between groups in proportions seropositive (and 95% confidence intervals [CIs] using the normal approximation to the sampling distribution of the difference) and reported the risk difference as a proportion. A risk difference of 0.08 would indicate that an additional 8% of individuals in the first comparison group were seropositive than in the second comparison group (eg, 88% vs. 80%). Immunogenicity data were stratified according to the conjugated molecule (PRP-HbOC, -OMP or -T). We report 1.0 μg/mL threshold data in figures in preference to 0.15 μg/mL threshold data because risk differences were generally larger at the higher threshold. We report GMC data where seropositivity data were not available. We did not assess the presence of small trials biases using funnel plots or the Egger test because few trials were available for most analyses.

RESULTS

The literature searches yielded a total of 4337 unique items; 4032 items from the 2010 database and 305 from reference lists or repeat database searches. Of these, 4299 items were excluded (Fig. 1). The remaining 38 items referred to 20 randomized or quasi-randomized trials reporting on eligible comparisons and outcomes. Included studies are described in Table 1 and Supplemental Digital Content 3, http://links.lww.com/INF/B614.[16–34] The 20 trials were conducted in 15 countries in Africa, Asia, Europe and North and South America. Sixteen trials used PRP-T, 3 used PRP-OMP and 2 used PRP-HbOC. One trial used PRP-T in 2 trial groups and PRP-HbOC in 2 other groups (Chile 1). Five trials did not report the number of individuals assigned to each intervention group. Where numbers were reported, a total of 6312 infants were assigned to intervention groups analyzed in this review: 661 infants to 2p+0 schedules, 1194 to 3p+0, 300 to 2p+1 and 4157 to 3p+1 schedules. The median number of participants in trials was 283 (range 54–1782).

Risk of Bias in Methods of Included Studies

Table 2 shows methodological features that could influence the risk of bias for the 20 trials. All trials individually assigned participants to intervention groups, and only 1 trial was judged to be quasi-randomized (USA 3). Allocation concealment was assessed as adequate in 2 trials and inadequate in 1 trial. In 17 trials, allocation concealment was not well enough described to be assessed. Outcome assessors (laboratory staff) were described as blinded in 11 of the 20 trials. Four trials reported mITT analyses (3 of which also conducted PP analyses but only stated that results were similar to mITT results), 9 reported PP analyses (2 of which also conducted mITT analyses but only stated that results were similar to PP results) and for 7 trials it was not clear which analysis was reported.

Head-to-head Comparisons Between Schedules

There were no eligible clinical or carriage outcome data from trials that compared different schedules of Hib vaccination. Twenty trials examined eligible schedule comparisons and presented seropositivity or GMC data. Nine of these provided data for comparisons of schedules with different numbers of doses in the primary or booster schedules and 14 of these provided data for comparisons of schedules with the same number of doses but different timings. Figures in Supplemental Digital Content 4 and 5, http://links.lww.com/INF/B615 and http://links.lww.com/INF/B616, show seropositivity (≥0.15 and ≥1.0 μg/mL) for all trial arms used in eligible comparative analyses.

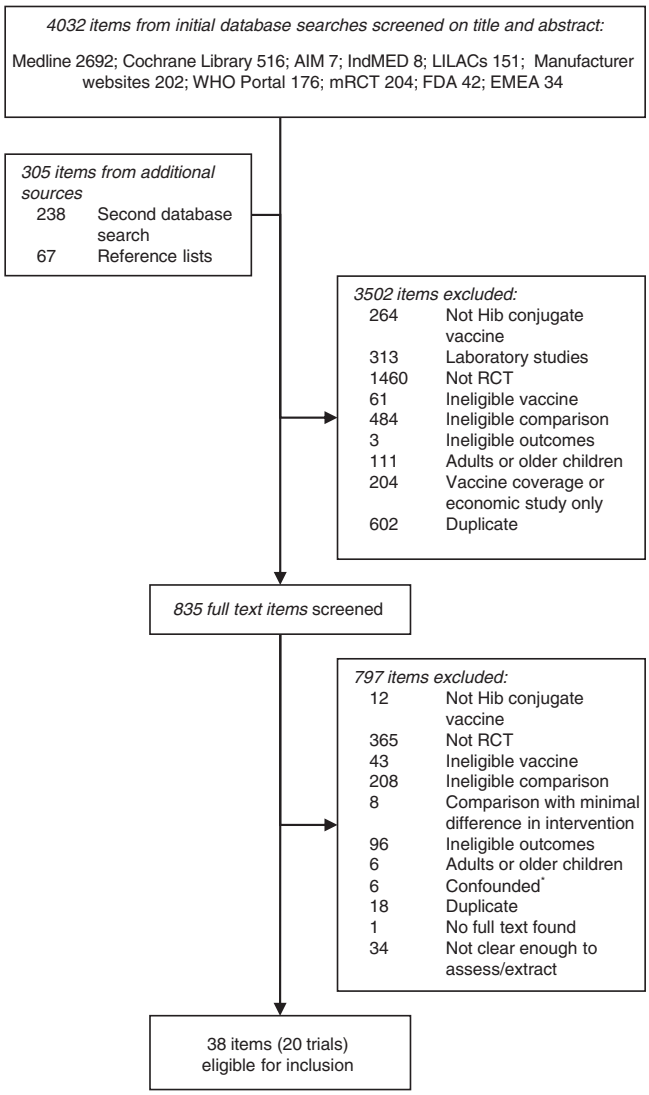

FIGURE 1. Flow diagram of studies. The 4032 items found in initial database searches include duplicates that were retrieved in 2 or more databases. *All 6 items relate to 1 trial where the only eligible outcomes were pneumonia and death, and children were randomized to either Hib and pneumococcal conjugate vaccine or to a malaria vaccine. Differences between groups could be due to Hib or pneumococcal vaccines.

Number of Doses in Primary and Booster Schedules, Immunological Data

3p+0 Versus 2p+0 Schedules. Seven trials provided data for this comparison (Chile 1, Chile 2, Guatemala, Netherlands, Niger, Sweden, USA 2). Six examined PRP-T and 2 examined PRP-HbOC (1 trial examined both). Six trials reported seropositivity (Chile 1, Chile 2, Guatemala, Netherlands, Niger, Sweden), and all trials reported GMC data.

Figure 2 shows the risk difference (≥1.0 μg/mL) for seropositivity between groups receiving 3p+0 and 2p+0 schedules for trials where the interval between the last dose and blood draw was the same for both arms. In 3 trials, examining PRP-T (Chile 1, Niger, Sweden), neither the 2p nor the 3p schedule was consistently favored and heterogeneity was high (I^2 90% at the 1.0 μg/mL threshold and 67% at the 0.15 μg/mL threshold, shortly after the last primary dose). By 6 months after the last primary dose, there was no difference between the schedules at the 1.0 μg/mL threshold (combined risk difference −0.02; 95% CI: −0.10, 0.06) and no heterogeneity (I^2 0%). Heterogeneity remained high 6 months after the last primary dose at the 0.15 μg/mL threshold (I^2 75%).

One trial (Chile 1) examined PRP-HbOC and presented seropositivity data. Point estimates favored the 3p group, but the confidence interval included the null effect. The trial which reported only GMC (USA 2) examined PRP-HbOC and compared a birth dose plus doses at 2 and 4 months of age with doses at 2 and 4 months of age. Two months after the last dose, the reported GMC in the 3p group (birth dose group) was 0.93 μg/mL (95% CI: 0.48, 1.69) and 0.20 μg/mL (95% CI: 0.10, 0.29) in the 2p group.

3p Versus 2p+1 Schedules. One trial (Sweden) using PRP-T provided data for this comparison. At 13 months of age (7 months after the 3p group received their last primary dose and 1 month after the 2p+1 group received their booster), the risk difference was −0.79 (95% CI: −0.87, −0.71) at the 1.0 μg/mL threshold and −0.20 (95% CI: −0.27, −0.13) at 0.15 μg/mL, favoring the 2p+1 schedule.

3p+1 Versus 2p+1 Schedules. Two trials using PRP-T provided data on seropositivity for this comparison (Netherlands, Sweden). Proportions seropositive 1 month after the booster vaccinations were high and similar in both groups. The combined risk difference was 0.01 (95% CI: −0.03, 0.05) at the 1.0 μg/mL threshold and 0.01 (95% CI: −0.01, 0.02) at 0.15 μg/mL with moderate (I^2 56%) and low (I^2 24%) heterogeneity, respectively.

3p+1 Versus 3p Schedules. Two trials examined PRP-T for this comparison (Canada 2, Europe). One reported seropositivity data (Europe) and both reported GMC. At 13 months of age (1 month after the 3p+1 group received their booster dose), the 3p+1 schedule resulted in higher seropositivity than the 3p schedule at both the 1.0 μg/mL (risk difference 0.59, 95% CI: 0.52, 0.67) and 0.15 μg/mL thresholds (risk difference 0.16, 95% CI: 0.11, 0.22). One trial reported only GMC (Canada 2). Multiple intervention groups in this trial were available for comparison and not all are presented here. At 16 months of age, the intervention group that received a 3p schedule with a booster dose at 15 months of age achieved a GMC of 29.92 μg/mL (95% CI: 24.58, 36.43, Canada 2) and a group which had received a 3p schedule with no booster dose by 16 months of age achieved a GMC of 0.32 μg/mL (95% CI: 0.25, 0.41, Canada 2).

Age at Start of Primary Schedule, Immunological Data

Eight trials compared schedules with the same number of doses, in which the first dose was given earlier or later (Belgium, Chile 2, China 1, China 2, Gambia 1, Gambia 2, Netherlands, Turkey). Seven examined PRP-T, and 1 examined PRP-OMP (Gambia 1). Seven trials reported seropositivity data, and 8 reported GMC. Seropositivity results at the 1.0 μg/mL threshold are shown in Figure 3. Some schedule comparisons differed in both the age at first dose and in the interval between doses in the primary schedule. There were only small differences in seropositivity between schedules and heterogeneity was low. The combined risk difference 1 month after the last primary dose was 0.02 (95% CI: −0.01, 0.05) at the 1.0 μg/mL threshold, based on 3 trials (I^2 1%). It was 0.01 (95% CI: 0.00, 0.02) at 0.15 μg/mL based on 4 trials (I^2 0%).

The trial which reported only GMC (Gambia 2) compared PRP-T doses at 2 and 4 months to doses at 1 and 3 months of age. One month after the last dose of vaccine, the GMC was 0.41 μg/mL (95% CI: 0.28, 0.61) in infants receiving the first dose at 2 months and 0.26 μg/mL (95% CI: 0.19, 0.35) in the group with the earlier

start. One study comparing a birth dose of PRP-HbOC plus doses at 2, 4 and 6 months of age with doses at 2, 4 and 6 months (USA 2) concluded that antibody levels were not higher after a birth dose.

Interval Between Doses, Immunological Data

Longer Versus Shorter Interval in Primary Schedules. Five trials provided immunological data comparing longer and shorter intervals in the primary schedule (Belgium, France, Turkey, USA 1, USA 3). Four trials compared 2-month intervals with 1-month intervals (Belgium, France, Turkey, USA 3); 3 used 3p schedules to PRP-T and reported both seropositivity and GMC data (Belgium, France, Turkey) and 1 used a 2p schedule with PRP-OMP and reported GMC data only (USA 3). At the 1.0 μg/mL threshold, neither the 2-month nor the 1-month interval schedule was consistently favored, but results were heterogeneous (Fig. 4). At the 0.15 μg/mL threshold, no difference was seen between the schedules and heterogeneity was low: the combined risk difference 1 month after the last primary dose was 0.00 (95% CI: −0.02, 0.02), I^2 0%. The trial using PRP-OMP (USA 3) was quasi-randomized, using alternation for assignment of interventions. The mean age at first vaccination was older in the 2-month interval group than in the 1-month interval group (4.1 and 3.2 months, respectively). Age-adjusted GMCs 1 month after the second vaccination were 3.95 μg/mL (95% CI: 2.63, 5.92) in the 2-month interval group and 2.32 μg/mL (95% CI: 1.48, 3.64) in the 1-month interval group. One trial compared 4-month intervals to 2-month intervals using PRP-OMP (USA 1), but results were difficult to interpret because the interval between vaccination and blood sampling differed between the groups being compared.

Longer Versus Shorter Interval Between Primary and Booster Schedules. Seven trials examined PRP-T and provided seropositivity and GMC data (Canada 1, Canada 2, Canada 3, Chile 2, China 1, Europe, France). There were no differences in seropositivity 1 month after the booster dose and little between-study heterogeneity. The combined risk difference was 0.00 (95% CI: −0.01, 0.01) at the 1.0 μg/mL threshold (Fig. 5) and 0.00 (95% CI: −0.01, 0.01) at 0.15 μg/mL, with I^2 14% and I^2 0%, respectively.

DISCUSSION

Immunological data in this systematic review showed few differences that were both consistent and clinically relevant between Hib conjugate vaccine schedules with 2 or 3 primary doses or between schedules with different intervals between doses. Participants who had received booster doses were more likely to be seropositive than those of the same age who had not. There is an absence of clinical outcome or nasopharyngeal carriage data in head-to-head comparisons of Hib schedules.

This study is, to our knowledge, the first systematic review to examine the evidence from head-to-head comparisons of different Hib conjugate vaccine schedules. The wide search means that relevant RCTs are unlikely to have been missed. We also attempted a detailed assessment of potential sources of heterogeneity and bias, but many trials were not reported completely enough for the risk

TABLE 1. Summary of Included Studies

Trial Name and Location	Conjugate Vaccine	Allocation Level	Schedules, Age at Administration in Months		Number of Participants Randomized	Immunological Outcomes Reported
			Intended	Actual, Mean (SD)		
Belgium[16]	PRP-T	Individual	3, 4, 5*	3.0 (0.1) 4.0 (0.1) 5.0 (0.2)	49†	Seropositivity GMC
			2, 4, 6*	2.1 (0.2) 4.0 (0.2) 5.9 (0.2)	54†	
Canada 1[17]	PRP-T	Individual	2, 4, 6 + b18	NR‡	82	Seropositivity GMC
			2, 4, 6 + b15		85	
			2, 4, 6 + b12		86	
Canada 2[18]	PRP-T	Individual	3p + b18	18.3 (0.3)	438	Seropositivity GMC
			3p + b17	17.4 (0.3)	450	
			3p + b16	16.4 (0.3)	449	
			3p + b15	15.4 (0.3)	445	
				Primary: NR		
Canada 3[19]	PRP-T	Individual	2, 4, 6 + b18	18.3 (0.3)	167	Seropositivity GMC
			2, 4, 6 + b15	15.3 (0.3)	168	
				Primary: NR		
Chile 1[20]	PRP-T	Individual	2, 4, 6	NR	78	Seropositivity GMC
			4, 6		79	
	PRP-HbOC		2, 4, 6		78	
			4, 6		78	
Chile 2[21]	PRP-T	Individual	3, 5, 7 + b12§	NR	710¶	Seropositivity GMC
			2, 4, 6 + b12§			
China 1[22]	PRP-T	Individual	3, 4, 5 + b18–20‖	NR	264	Seropositivity GMC
			2, 3, 4 + b18–20‖		264	
China 2[23]	PRP-T	Individual	3, 4, 5**	3.3 (0.3)	324	Seropositivity GMC
			2, 3, 4**	2.3 (0.3)	330	
				Dose 2–3: NR		
Europe[24] (Austria, Germany, Greece)	PRP-T (booster)††	Individual	3p + b13‡‡	NR	220	Seropositivity GMC
			3p + b12‡‡	14.9 (3.2)	224	
				Primary NR		
France[25]	PRP-T	Individual	2, 4, 6 + b15–17	NR	258	Seropositivity GMC
			2, 3, 4 + b15–17		258	

(*Continued*)

TABLE 1. Continued

Trial Name and Location	Conjugate Vaccine	Allocation Level	Schedules, Age at Administration in Months		Number of Participants Randomized	Immunological Outcomes Reported
			Intended	Actual, Mean (SD)		
Gambia 1[26]	PRP-OMP	Individual	2, 4	NR§§	95	Seropositivity
			1, 3		99	GMC
Gambia 2[27]	PRP-T	Individual	2, 4	NR	43	GMC
			1, 3		45	
Guatemala[28]	PRP-T	Individual	2, 4, 6	NR	325	Seropositivity
			7, 9		106	GMC
Netherlands[29]	PRP-T	Individual	3, 4, 5 + b11¶¶	NR	181	Seropositivity
			6, 7 + b13¶¶		182	GMC
Niger[30]	PRP-T	Individual	1.5, 2.5, 3.5	All groups, mean (range):	59	Seropositivity
			2.5, 3.5	1.9 (0.9–2.8)	62	GMC
				3.0 (2.1–5.1)		
				4.2 (3.0–6.8)		
Sweden[31]	PRP-T	Individual	2, 4, 6 + b13	NR‖‖	118	Seropositivity
			3, 5 + b12		118	GMC
Turkey[16]	PRP-T	Individual	3, 4, 5*	3.0 (0.1)	78†	Seropositivity
				4.0 (0.2)		GMC
				5.1 (0.3)		
			2, 4, 6*	2.1 (0.2)	81†	
				4.0 (0.3)		
				5.9 (0.3)		
USA 1[32]	PRP-OMP	Individual	2, 6	NR	36***	Seropositivity
			2, 4		39***	GMC
USA 2[33]	PRP-HbOC	Individual	2, 4, 6	NR†††	150‡‡‡	GMC
			0, 2, 4, 6			
USA 3[34]	PRP-OMP	Individual	2–6, 4–8	4.1 (1.6)	27	GMC (adjusted)
				6.1 (1.6)		
			2–6, 3–7	3.2 (1.3)	27	
				4.2 (1.3)		

All times are in months of age unless otherwise stated. One reference is supplied for each trial in this table. A complete list of references for each trial can be found in Supplemental Digital Content 3, http://links.lww.com/INF/B614.

*Multiple groups provide this comparison for this trial. Results presented compare a group receiving PRP-T and DTaP in separate syringes at 3, 4, 5 months with a group receiving PRP-T and DTaP in separate syringes at 2, 4, 6 months. Another group receiving PRP-T at 3, 4, 5 months in the same syringe as DTaP.

†Number receiving vaccine; number randomized not reported.

‡Ages not stated, but the following information is given for the booster doses: "The intended schedule of immunization was met for each child with single exceptions at 15 months (one week late) and 18 months (2 weeks late)."

§Multiple groups provide this comparison for this trial. Results presented compare a group receiving PRP-T at 3, 5, 7 months and DTaP combined with IPV at 2, 4, 6 months with a group receiving PRP-T at 2, 4, 6 months and DTaP combined with IPV at 2, 4, 6 months in another limb. Other groups receiving PRP-T at 3, 5, 7 months either received oral polio vaccine instead of IPV or had DTaP and IPV given as separate injections. The other group receiving PRP-T at 2, 4, 6 months received PRP-T in the same syringe as DTaP and IPV.

¶Number randomized to each group not reported. Seven hundred ten infants randomized to 5 groups (not all included here).

‖Multiple groups provide this comparison for this trial. Results presented compare a group receiving PRP-T, IPV and DTaP in the same syringe at 3, 4, 5 months with a group receiving PRP-T, IPV and DTaP in the same syringe at 2, 3, 4 months. Another group receiving PRP-T at 3, 4, 5 months received DTaP and IPV separately at the same time (ie, 3 separate syringes).

**Multiple groups provide this comparison for this trial. Results presented compare a group receiving PRP-T, IPV and DTaP in the same syringes at 3, 4, 5 months with a group receiving PRP-T, IPV and DTaP in the same syringes at 2, 3, 4 months. Another group receiving PRP-T at 2, 3, 4 months received DTaP in the same syringe and IPV at the same time but in a separate syringe.

††Type of conjugate vaccines for the primary series was not specified in this trial.

‡‡Multiple groups exist for the 3p + b12 schedule in this trial. Results presented compare a group receiving 3p then meningococcal conjugate vaccine at 12 months and PRP-T at 13 months with a group receiving 3p then PRP-T at 12 months.

§§Ages not stated, but the following information is given: "Full compliance with the vaccination schedule and blood sampling was achieved by 85 infants in group A (immunized with two doses of vaccine at 1 and 3 months) and by 56 in group B (immunized at 2 and 4 months)."

¶¶Multiple groups provide this comparison for this trial. Results presented compare a group receiving PRP-T at 3, 4, 5 + b11 months and diphtheria, tetanus, whole-cell pertussis vaccine combined with IPV as a separate injection from PRP-T at 3, 4, 5 + b11 months with a group receiving PRP-T at 6, 7 + b13 months and diphtheria, tetanus, whole-cell pertussis vaccine combined with IPV (not with PRP-T) at 3, 4, 5 + b11 months. The other group receiving PRP-T at 3, 4, 5 + b11 months received PRP-T in the same syringe as diphtheria, tetanus, whole-cell pertussis vaccine and IPV.

‖‖Ages not stated, but most doses were given on time: "805 injections were administered. Seven injections were given 1 to 6 days out of time range, 2 injections were given >1 month out of time range."

***Number analyzed; number of randomized or immunized children not reported.

†††The group receiving 2, 4, 6 PRP-HbOC received the 3rd dose at a mean age of 6.7 months. Other groups and doses not reported.

‡‡‡Total recruited, randomized and immunized; numbers per group not reported.

3p indicates 3-dose primary schedule where intended ages at vaccination not specified; +b, booster dose given at number of months indicated. DTaP, diphtheria, tetanus, acellular pertussis vaccine; Hib, *Haemophilus influenzae* type b vaccine; IPV, inactivated polio vaccine; Men A and C vaccines, conjugate or polysaccharide meningococcal A and C vaccines; NR, not reported; p, primary course; PRP, polyribosylribitol phosphate; PRP-HbOC, polyribosylribitol phosphate conjugated to diphtheria toxin CRM197; PRP-OMP, polyribosylribitol phosphate conjugated to outer membrane protein of *Neisseria meningitidis*; PRP-T, polyribosylribitol phosphate conjugated to tetanus toxoid; SD, standard deviation.

of bias to be assessed. A limitation identified by this review was the paucity of data on several outcomes and comparisons of interest. We did not include data from observational studies because well-conducted RCTs are at lower risk of bias than observational study designs,[35,36] and because observational studies have been summarized elsewhere.[37,38] The potential for bias does remain in many of the included trials, with allocation concealment, blinding of outcome assessors and exclusions after randomization being key trial design features influencing the risk of bias within trials.[39] In particular, many trials in this review explicitly excluded some randomized

TABLE 2. Methodological Features of Trials

Study, Vaccine (Manufacturer)	Adequate Randomization Sequence Generation	Adequate Randomization Allocation Concealment	Blinding of Patient or Parent to Exposure Status	Blinding of Outcome Assessors (Immunological Outcomes)	Blinding of Other Persons	Modified Intention-to-treat or PP Analyses, Immunological Outcomes
Belgium[16]	Unclear, randomization list but generation not reported	Unclear, not reported. Allocated "according to a randomization list and following chronological order of enrolment in the trial"	No, not possible due to schedule differences	Yes	Unclear, not reported	mITT (PP performed and "similar")
Canada 1[17]	Yes, computer-generated list of random numbers	Unclear, sealed, serially numbered envelopes that were opened in sequence, but not stated if opaque	No, not possible due to schedule differences	Unclear, authors refer to "code-numbered samples," but no explicit description of blinding	Not reported	mITT
Canada 2[18]	Unclear, not reported	Unclear, not reported	No, not possible due to schedule differences	Unclear, trial described as open-label	Unclear, trial described as open-label	PP (mITT performed and "similar")
Canada 3[19]	Unclear, not reported	Unclear, not reported	Parents partially blinded. Not blinded to age at vaccination	Unclear, not reported	Unclear, not reported	Unclear
Chile 1[20]	Unclear, not reported how "list of correlative numbers" generated	Unclear, not well reported	No, not possible due to schedule differences	Yes	Vaccinators not blinded	Unclear
Chile 2[21]	Unclear, does not report how "list of ... study numbers, in blocks of 10" generated	Unclear, not reported	No, not possible due to schedule differences	Yes	Unclear, trial reported to be "open"	mITT (PP analysis conducted with "identical results")
China 1[22]	Unclear, not reported	Unclear, not reported	No, not possible due to schedule differences	Yes	Unclear, trial reported to be "open"	Unclear
China 2[23]	Unclear, not reported	Unclear, not reported	No, not possible due to schedule differences	Unclear, trial reported to be "open"	Unclear, trial reported to be "open"	PP
Europe[24] (Austria, Germany, Greece)	Unclear, not reported	Unclear, not reported	No, not possible due to schedule differences	Unclear, trial reported to be "open"	Unclear, trial reported to be "open"	PP
France[25]	Unclear, not reported	Unclear, not reported	Unclear, but unlikely due to schedule differences	Unclear, trial reported to be "open"	Unclear, trial reported to be "open"	PP (mITT performed and reported to be consistent with PP)
Gambia 1[26]	Unclear, "using a system of random numbers"	Yes, on-site computer system, with automated and consecutive allocation of vaccination codes corresponding to coded vials	No, not possible due to schedule differences	Yes	Field workers not blinded	PP

(*Continued*)

TABLE 2. Continued

Study, Vaccine (Manufacturer)	Adequate Randomization Sequence Generation	Adequate Randomization Allocation Concealment	Blinding of Patient or Parent to Exposure Status	Blinding of Outcome Assessors (Immunological Outcomes)	Blinding of Other Persons	Modified Intention-to-treat or PP Analyses, Immunological Outcomes
Gambia 2[27]	Unclear, "system of random numbers incorporated into a computerized call program"	Yes, on-site computer system, with automated and consecutive allocation of vaccination codes corresponding to coded vials	No, not possible due to schedule differences	Yes, laboratory staff blinded	Unclear, not reported	Unclear
Guatemala[28]	Yes, computer-generated random numbers	Unclear, sequentially numbered sealed envelopes. Not stated if opaque or if linked to individuals before opening	Unclear, trial reported to be "open"	Unclear, trial reported to be "open"	Described as "open study"	Unclear
Netherlands[29]	Yes, computer generated list	Unclear, not reported	Unclear, not reported	Yes	Unclear, not reported	PP
Niger[30]	Unclear, not reported	Unclear, not reported	Unclear, not reported	Unclear, "assays were performed on coded specimens," but no additional description given	Those who assess adverse events were blinded	Unclear
Sweden[31]	Unclear, "randomly assigned, in blocks of 10," but sequence generation not reported	Unclear, not reported	No, not possible due to schedule differences	Yes	Unclear, trial reported as "open"	PP
Turkey[16]	Unclear, randomization list but generation not reported	Unclear, not reported. Allocated "according to a randomization list and following chronological order of enrolment in the trial"	No, not possible due to schedule differences	Yes	Unclear, not reported	mITT (PP performed and "similar")
USA 1[32]	Unclear, site-specific randomization lists but generation not reported	Unclear, Vials supplied only with a code number but not reported if vials were identical in appearance. Unclear who randomized the infants	Yes, placebo used	Yes	"Investigators who enrolled, interviewed, or evaluated subjects or parents were blinded to study group assignment"	PP
USA 2[33]	Unclear, not reported	Unclear, not reported	Yes	Yes	Vaccinators not blinded. Those assessing safety were blinded	Unclear
USA 3[34]	No, alternation	No, alternation	No, not possible due to schedule differences	Unclear, not reported	Unclear, not reported	PP

All assessments based on information contained in published articles or prepublication articles. Authors of individual trials were not contacted for information on methodological features. One reference is supplied for each trial in this table. A complete list of references for each trial can be found in Supplemental Digital Content 3, http://links.lww.com/INF/B614.

mITT indicates modified intention-to-treat analysis, similar to an intention-to-treat analysis but with some modifications to inclusion criteria such as excluding those who did not receive a first dose of vaccine; PP, per-protocol analysis, analysis where individuals with protocol violations (such as not receiving the intended vaccination schedule) are excluded.

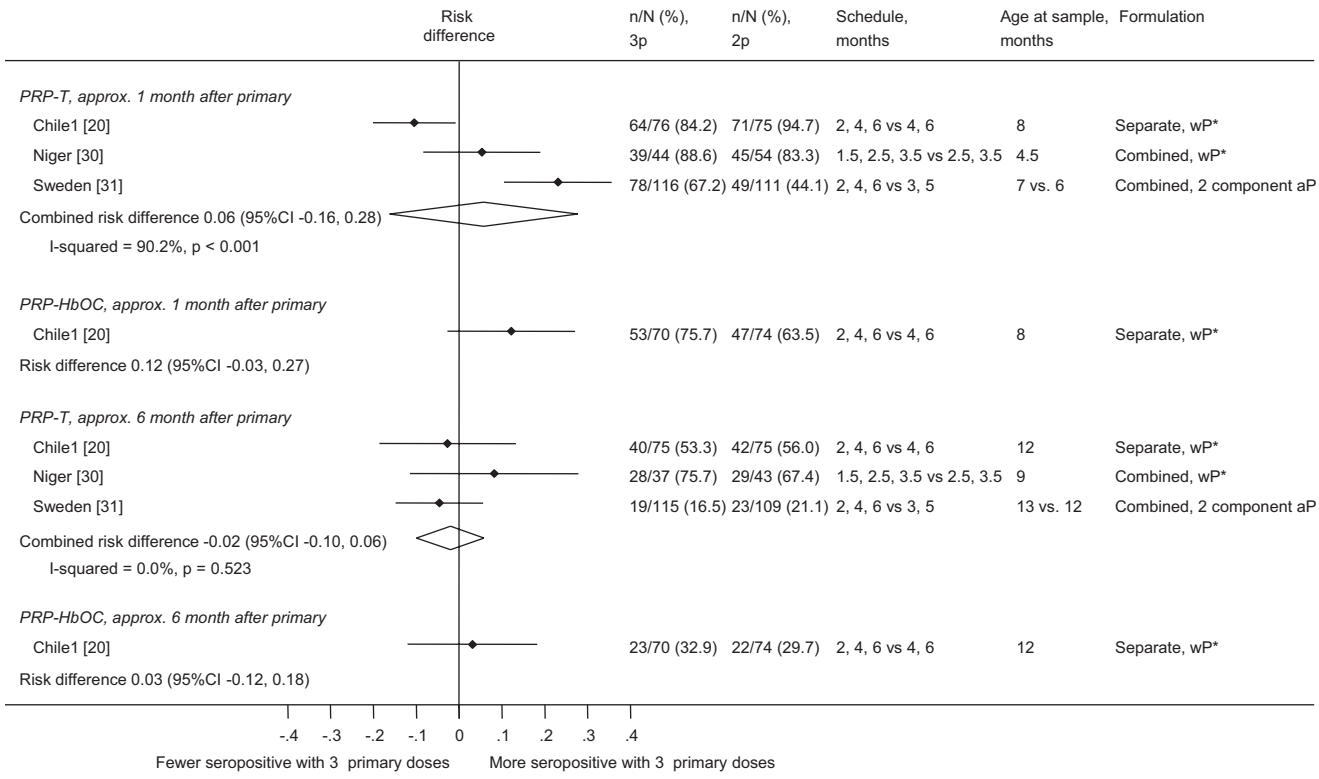

FIGURE 2. Comparison of seropositivity after 3 or 2 primary doses of Hib conjugate vaccine, 1.0 μg/mL. Additional data for this comparison are not shown on this plot because the interval between the last dose of vaccine and blood sampling differed between the groups being compared within each study, making the comparison unfair. These data came from Chile 2 (at 1 or 2 months after the primary dose), Guatemala (at 3 or 6 months after the primary dose) and Netherlands (4 or 6 months after the primary dose). Combined indicates Hib vaccine administered in the same syringe as pertussis containing vaccine; separate indicates Hib vaccine administered by itself, either at the same time as or at different times from other vaccines; aP, acellular pertussis vaccine; wP, whole-cell pertussis vaccine. *Not specified as whole-cell pertussis vaccine but assumed to be whole cell due to the year in which the trial was conducted.

individuals by conducting only a PP analysis. For some design features, it is difficult to categorize the risk of bias if the design feature is poorly described. For example, an incomplete description of allocation concealment could be compatible with either a high or low risk of bias; if allocation was adequate, the risk of bias is low but if allocation concealment was not well conducted, bias might occur if it can be easily predicted which individuals are more or less likely to seroconvert. Incomplete descriptions for features such as blinding are less important when considering immunological results where outcomes are assessed by laboratory technicians. It is possible and even likely that outcome assessors were blinded, even if this was not reported. Even if the laboratory staff are not blinded, automated procedures are likely to reduce the risk of bias.

The immunological data from available trials do not clearly favor either a 2-dose or a 3-dose primary schedule. There were also no important differences in seropositivity for PRP-T schedules starting at either 2 versus 3 months or PRP-OMP schedules starting at 1 versus 2 months of age. Available clinical data show good protection against invasive Hib disease with 2p+0 schedules using PRP-OMP,[40] and with 3p+0 schedules using PRP-T or PRP-HbOC,[40–44] when compared with no Hib vaccine, and these data have been summarized several times.[6–9] However, estimates of vaccine efficacy from different trials cannot be compared directly as evidence of equivalence or superiority of 1 particular schedule, and there are too few trials for a network meta-analysis, which would allow such a comparison.[45,46]

Two-month intervals between doses in the primary schedule were not shown to be consistently more immunogenic than 1-month intervals. Meta-analyses either showed marked heterogeneity or showed little heterogeneity and no difference between 2- and 1-month intervals. It is challenging to draw conclusions about clinical efficacy based on immunological findings because the clinical relevance of Hib seropositivity levels and GMCs are not well established in general,[10] and also because of differences in the schedules compared within each study other than the difference of interest. Data from an observational review found no strong evidence from cohort or case-control studies that the choice of intended intervals of 1 or 2 months between doses affects vaccine effectiveness,[38] but differences between the intended and actual schedules and other factors such as herd immunity in the population again add complexity to interpretation.[5]

A booster dose after a primary series of either 2 or 3 doses of Hib conjugate vaccine results in high levels of seropositivity. There was no evidence from trials that the age at which the booster dose is given or the interval between the primary series and the booster dose affects the level of seropositivity. Seropositivity levels in children after a booster dose are much higher than in children who received the same primary schedule without a booster. The interval between the last vaccine dose and blood draw is, however, shorter in children receiving the booster than in those who received only the primary schedule, and it is not clear if differences in antibody levels can be interpreted as differences in protection from Hib disease.[10]

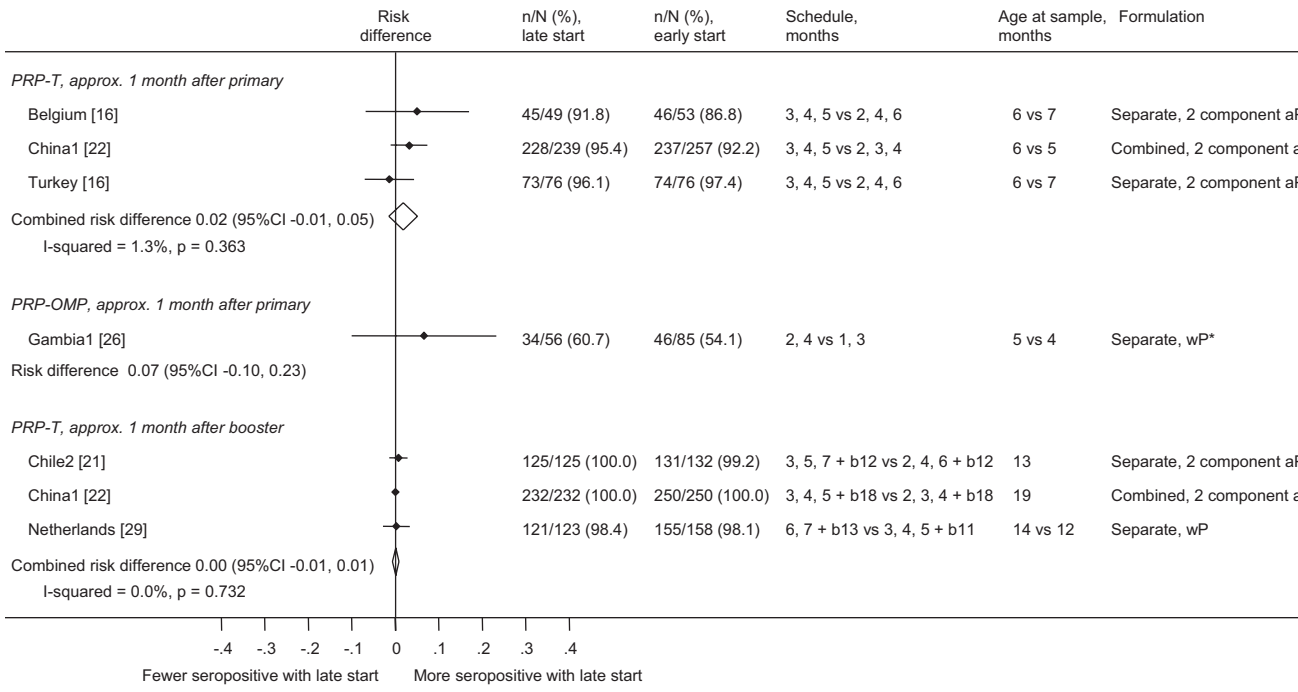

FIGURE 3. Comparison of seropositivity after late or early start of primary course of Hib conjugate vaccine, 1.0 μg/mL. Additional data for this comparison are not shown on this plot because the interval between the last dose of vaccine and blood sampling differed between the groups being compared within each study, making the comparison unfair. These data came from China 1 (at 13 or 14 months after the primary dose), Gambia 1 (at 14 or 15 months after the primary dose) and Netherlands (4 or 6 months after the primary dose). Combined indicates Hib vaccine administered in the same syringe as pertussis containing vaccine; separate indicates Hib vaccine administered by itself, either at the same time as or at different times from other vaccines; aP, acellular pertussis vaccine; wP, whole-cell pertussis vaccine. *Not specified as whole-cell pertussis vaccine but assumed to be whole cell due to the year in which the trial was conducted.

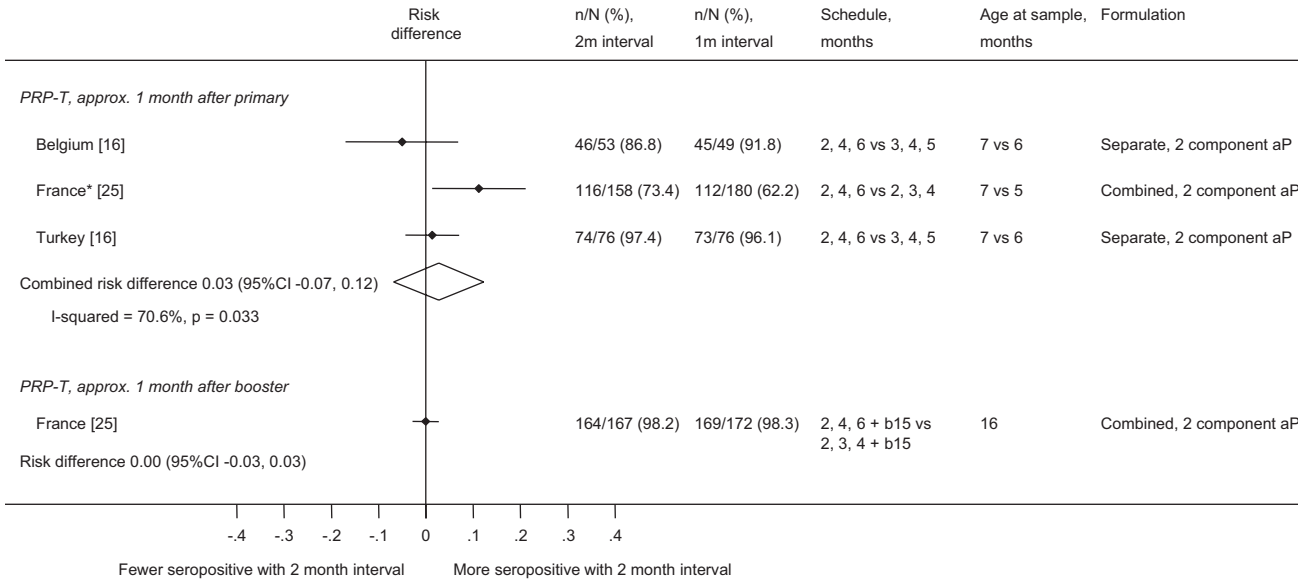

FIGURE 4. Comparison of seropositivity after 2- or 1-month intervals between doses in the primary course of Hib conjugate vaccine, 1.0 μg/mL. Additional data for this comparison are not shown on this plot because the interval between the last dose of vaccine and blood sampling differed between the groups being compared within each study, making the comparison unfair. These data came from France (at 9 or 11 months after the primary dose). Combined indicates Hib vaccine administered in the same syringe as pertussis containing vaccine; separate indicates Hib vaccine administered by itself, either at the same time as or at different times from other vaccines; aP, acellular pertussis vaccine; wP, whole-cell pertussis vaccine. *Data for this trial reported unclearly at this time point and for this definition of seropositivity.

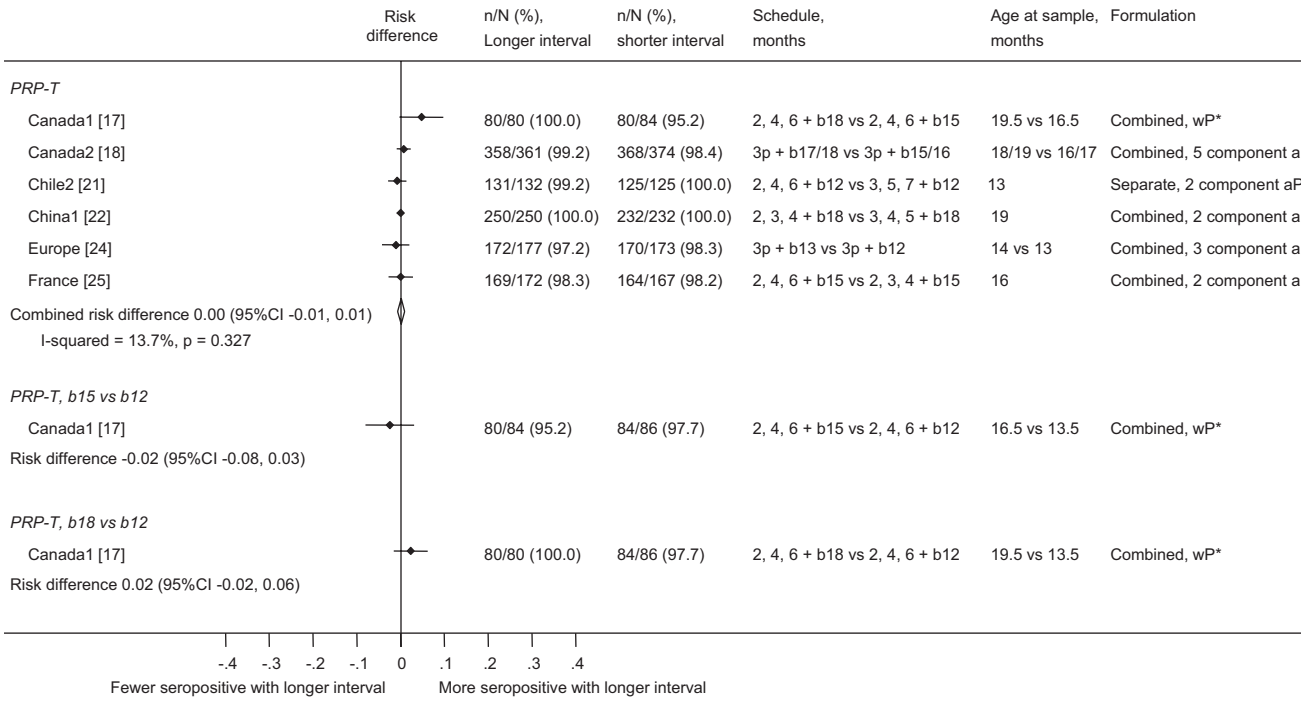

FIGURE 5. Comparison of seropositivity after long or short intervals between primary and booster doses of Hib conjugate vaccine, 1.0 μg/mL. Combined indicates Hib vaccine administered in the same syringe as pertussis containing vaccine; separate indicates Hib vaccine administered by itself, either at the same time as or at different times from other vaccines; aP, acellular pertussis vaccine; wP, whole-cell pertussis vaccine. *Not specified as whole-cell pertussis vaccine but assumed to be whole cell due to the year in which the trial was conducted

This review was not designed to collect data about antibody persistence, and therefore, caution should be taken when examining such data from this review. However, when data from individual groups in trials eligible for this review are plotted alongside each other (Figs., Supplemental Digital Content 4 and 5, http://links.lww.com/INF/B615 and http://links.lww.com/INF/B616), it can be seen that the proportion seropositive tends to be higher soon after a booster dose than soon after the last primary dose, or several months after the last primary dose, particularly at the 1.0 μg/mL threshold. Trials that assessed seropositivity more than 1 month after the last primary dose showed generally lower proportions seropositive than those assessing seropositivity 1 month after the last primary dose. In the 1 trial with long follow up after a booster dose, a high proportion of individuals remained seropositive at the 0.15 μg/mL threshold years after the booster dose and a much lower proportion at the 1.0 μg/mL threshold. These trends are in general agreement with studies that have found sustained antibody persistence after a booster dose.[47,48] The United Kingdom experienced an increase in Hib cases several years after an initial decline in cases subsequent to the introduction of a 3p+0 schedule (2, 3, 4 months) alongside an early catch-up campaign. Cases again declined after 2 booster campaigns and the introduction of a routine booster dose to the vaccine schedule.[49] However, the situations in which a booster dose should be used remain unclear and might relate to local epidemiology, coadministered vaccines and the potential for natural boosting as well as other factors.[50,51]

This review did not aim to examine the effects of coadministrated vaccines on Hib conjugate vaccine efficacy, which is best examined in trials comparing groups with different coadministered vaccines but with the same schedule. However, conclusions from our review about the relative effects of different schedules do not change when restricted to trials that coadministered acellular pertussis vaccine or trials that coadministered whole-cell pertussis vaccine. In analyses that included both trials in which whole-cell pertussis vaccine was coadministered and trials in which acellular pertussis vaccine was coadministered, the relative effects of different schedules of Hib vaccine did not appear to change substantially between studies. However, owing to the limited availability of data in each analysis, this could not be formally assessed using statistical methods such as meta-regression. The observational review conducted simultaneously with our review found no strong evidence from cohort studies that coadministration with acellular pertussis vaccine–reduced vaccine effectiveness, but 2 case-control studies conducted in the United Kingdom provided some evidence of a reduction.[38,51,52] Further carefully conducted systematic reviews of RCTs, as well as observational data, could provide useful information about this and other questions about Hib vaccine scheduling.

Hib conjugate vaccine 2p+1, 3p+0 and 3p+1 schedules are all likely to provide protection against Hib disease and, until further data about the relative effects of different Hib vaccine schedules are available, the choice of schedule is likely to depend on the setting. For example, in settings where the burden of severe Hib disease lies with children under 1 year of age, it might be more appropriate to provide 3 doses of Hib vaccine early in life. In settings where the disease burden occurs later, or where a resurgence of Hib cases is seen after the introduction of Hib vaccine, it might be advantageous to use a schedule where the third dose is given as a booster. Programmatic considerations are also likely to influence the choice of Hib vaccine schedule. Costs of vaccine administration are likely to be lower and vaccine coverage higher if vaccine administration

is combined with other routine scheduled health visits. Additionally, most Hib vaccines are administered as combined vaccines, which means that the scheduling of the other coadministered vaccines must also taken into account when choosing a Hib vaccine schedule.

Future decisions relating to Hib vaccination could be informed by well-conducted RCTs with head-to-head comparisons of schedules that collect data on clinical outcomes. Trials comparing schedules would need to be extremely large to provide sufficient statistical power to show difference between schedules, but trials of this type have been conducted for other vaccines.[53]

Variation in the burden of disease, health infrastructure and scheduling of other vaccines creates complexity in determining optimal vaccination schedules. Thus, information on the benefits of different vaccine schedules is essential if informed decisions are to be made. In this comprehensive systematic review, we highlight the absence of clinical and carriage data from trials comparing Hib vaccine schedules and scarce immunological data from such comparisons. We show there is no clear evidence from vaccine trials that any 2p+1, 3p+0 or 3p+1 schedule of Hib conjugate vaccine is likely to provide better protection against Hib disease than other schedules. Until additional data about the relative effects of different Hib vaccine schedules are available, the choice of Hib vaccination schedule is likely to be determined by the epidemiological and programmatic conditions in individual settings.

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